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WHOLE BODY BUFFERS IN THE REGULATION OF ACID-BASE EQUILIBRIUM

Twenty-five years ago John P. Peters with Donald D. Van Slyke wrote one of the most precise, lucid, and comprehensive accounts of acid-base equilibrium that has ever appeared in scientific literature, namely, Chapter 18 in Volume I, *Interpretations,* of *Quantitative clinical chemistry.*[44] This account rested on the basic experimental work of Van Slyke and other investigators and especially on the classic work of Lawrence J. Henderson[30, 31] who in 1908 first delineated in a unified manner the physicochemical and physiological mechanisms for the maintenance of neutrality of the fluids of the body. In the quarter of a century since the appearance of *Quantitative clinical chemistry,* although much research activity has taken place in this field, no major revision has had to be made of the fundamental concepts as set forth in that work. Nevertheless, the investigative efforts of this subsequent period have greatly enhanced our understanding of both the physicochemical and the physiological mechanisms involved. This is true especially as regards (i) the linked transfers of ions which participate in the functioning of buffer systems, (ii) the rôle of the kidney in acid-base regulation, and (iii) the relationship of disease processes to disturbances of acid-base equilibrium. The first of these three categories is the subject of this paper; the other two are treated in part in some of the following contributions.

The first defense of body fluid neutrality is the physicochemical mechanism of the buffer systems. The buffer systems of the body consist of weak acids and their salts which react with strongly dissociated acids to form a neutral salt and a slightly dissociated weak acid, thus minimizing changes in hydrogen ion concentration. These systems are principally bicarbonates, phosphates, and proteins, and function according to circumstances as either donors or acceptors of hydrogen ions or protons. Once these buffer systems had been identified by Henderson, the attention of investigators was focussed primarily on the buffering activity of the blood.[32, 53, 21, 55] This is easily understandable since blood is such a readily accessible portion of the

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body fluids. On the other hand, studies of buffers in other phases or tissues have not been entirely wanting. Earlier investigators of this aspect of the problem were concerned: (i) with the amount of exogenous acid or alkali which could be tolerated or buffered by the living body or the amount of "nonmetabolic" carbon dioxide* which could be extracted or retained, and (ii) with the extent to which other tissues shared this buffering activity with the blood. More recently, with a greatly expanded knowledge of ionic transfers between multiple phases of the body fluids, investigators have become interested in the quantitative aspects of these ionic exchanges as they relate to whole body buffering of acid-base disturbances.

EARLIER STUDIES OF TISSUE BUFFERING

Henderson[30] clearly stated in 1908 the concept of multiple buffer systems in heterogeneous phases of the body fluids:

> Thus it appears that, through the nature of carbonic acid, and the fact that there is in the body a nearly constant supply of it, this substance possesses a quite wonderful capacity, more than three times as great as any other substance not thus regulated by a constant supply could have, of preserving the reaction in the neighborhood of a hydrogen ion concentration 0.3×10^{-7}.
>
> . . . When, finally, in the process of neutralizing acid, the bicarbonates have been nearly used up, and the reaction is almost precisely at the neutral point, it is evident that protoplasm still possesses in the phosphates substances which have, thanks to the ionization constant of the ion H_2PO_4 (2×10^{-7}), nearly the greatest efficiency which ordinary substances could possess in checking increase of acidity. Moreover the diffusibility† of acid phosphates probably adds to their efficiency, much as the adjustment of carbonic acid tension does to that of the bicarbonates.
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> ... The above considerations show that in the matter of neutrality regulation the single phase equilibrium in the body is rendered far more efficient than it could otherwise be by the interventions of other phases acting selectively as reservoirs of supply and as vehicles of escape. Thus the theoretical considerations above developed regarding neutrality regulation must be modified for a heterogeneous system, in so far as relative efficiency of different substances is concerned; for, by the intervention of other phases, substances not possessing the highest efficiency in this process in a single phase system, may become enormously more efficient than any closed single phase system easily surpassed by the heterogeneous system of the body.

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* Although all, or essentially all, carbon dioxide in the body is metabolic in origin, "nonmetabolic" carbon dioxide refers to that portion which is present in the several phases of the body fluids, chiefly in the form of bicarbonate, and which is in dynamic equilibrium with the stream of "metabolic" carbon dioxide steadily flowing from its cellular sources to the organs of excretion. Transfers of hydrogen or of bicarbonate ions, as discussed in this paper, refer, therefore, to shifts in this dynamic equilibrium and not to alteration in a static ionic pattern.

† Henderson includes in this term the renal excretion of monobasic phosphate.

And thus Lawrence Henderson clearly formulated a concept of acid-base regulation which was to be so well supported by the experimental work of the next fifty years.

Investigators of the subject of whole body buffering, following Henderson's formulation, were concerned first with the effects of the *addition of strong acids or alkalies* to the organism (Table 1). Van Slyke and Cullen[61] injected dilute sulphuric acid into dogs by the intravenous route and calculated that five-sixths of the acid was neutralized by buffers outside the blood. Palmer and Van Slyke[43] administered sodium bicarbonate by mouth to human subjects and estimated that its volume of distribution was equivalent, on the average, to 70 per cent of the body weight, i.e., the alkali was buffered in part by systems outside the blood and interstitial fluid. Haldane[29] studied the distribution of ingested ammonium chloride, a "metabolic" acid, by observing its effect on the content of carbon dioxide in blood; he concluded that only about one-eighth of the retained "acid" could be accounted for in an assumed blood volume of five liters. The same problem was approached in a different experimental manner by Banus and Katz;[4] these workers perfused the hind leg of a dog with blood strongly acidified with dilute hydrochloric acid. Since the perfusion resulted in a rise in CO_2 combining power of the blood from the initially depressed levels, without a change in chloride concentration, it was concluded that tissues in the hind leg buffered the acidified blood by at least one of three mechanisms: (i) shift into tissues of an anion other than chloride, (ii) shift out of tissues of cation, or (iii) increase in the protein buffers of the blood. Thus, by 1927 Henderson's concept of heterogeneous phase buffering received ample experimental confirmation in respect to so-called "metabolic" disturbances of acid-base equilibrium.

Investigation of the capacity of tissues and the whole body *to retain or to surrender nonmetabolic carbon dioxide or carbonic acid* when the alveolar CO_2 pressure was experimentally raised or lowered, extended the confirmation of Henderson's concept to "respiratory" disturbances of acid-base equilibrium (Table 2). In 1926 Shaw[52] subjected cats to inhalation of 7.5 per cent CO_2 and found that 84 to 92 per cent of the nonmetabolic CO_2 absorbed was retained in mixed tissues outside a blood volume assumed to equal 5.5 per cent of the body weight. Brocklehurst and Yandell Henderson[10] studied the effects of CO_2 inhalation and hyperventilation in human subjects and calculated that approximately one-half of the capacity of the body to retain or to give up CO_2 lay in the blood. The short duration of their experiment (two to three minutes) may explain the discrepancy between their findings and those of other workers. Adolf, Nance, and Shiling[1] repeated the experiment of CO_2 inhalation for periods of 30 minutes and

TABLE 1. DISTRIBUTION OF WHOLE BODY BUFFERING OF ACUTE EXPERIMENTAL METABOLIC DISTURBANCES OF ACID-BASE EQUILIBRIUM

Investigators	*Experimental procedure*		*No. Expts.*	*Species*	*Av. distribution of buffering*			*Assoc. "intracellular" ionic transfers*—av.*			
					Intravascular (red cell + plasma)	*Extravascular*					
						Interstitial	*"Intracellular"*	*Na*	*K*	*Anion*	*Extracellular reference space*
					per cent of total body buffering			equivalent per cent of total body buffering			
A. Metabolic Alkalosis											
Palmer and Van Slyke[48]	$NaHCO_3$	p.o.	9	Man	13‡		87				
Axelrod, Seip, and Pitts[3]	"	i.v.	6	Dog			43	+18	0	—25 (Cl)	Inulin
van Goidsenhoven, Gray, Price, and Sanderson[60]	"	p.o.	3	Man				+13		—37 (Cl)	Inulin
Singer, Clark, Barker, Crosley, and Elkinton[54]	"	i.v.	7	Man	26	44	30	+35	—15		Chloride
B. Metabolic Acidosis											
Van Slyke and Cullen[61]	H_2SO_4	i.v.	1	Dog	17‡		83				
Haldane[29]	NH_4Cl	p.o.	6	Man	13‡		87				
Schwartz, Jensen, and Relman[48]	NH_4Cl	p.o.†	3	Man	16	29	55	—22	—34		Chloride
Swan and Pitts[58]	HCl	i.v.	5	Dog	18	25	51	—36	—15		Radiosulfate
Tobin[59]	HCl	i.v.	7	Cat				—35	—5	+20 (Cl)	Inulin
								—58	—6		Chloride

The data in this table and in Table 2 have been recalculated in part for purposes of comparison.

* Exclusive of the red-cell phase.

† Subjects depleted of sodium prior to experiment.

‡ See footnote † in Table 2.

calculated that 82 to 97 per cent of the CO_2 "capacity" lay in the tissues outside of the blood. Irving, Ferguson, and Flewes[34] analyzed the CO_2 content of muscle and bone, as well as of blood, from cats subjected to high and low respiratory levels of CO_2; no change in the fairly high CO_2 content of bone was observed and muscle plus blood accounted for only 10 per cent of the nonmetabolic CO_2 lost and only 20 per cent of that retained. Rosenbaum,[46] working in John P. Peters' laboratory, examined the volume of distribution of nonmetabolic CO_2 in human subjects as compared with that of an exogenous "extracellular" tracer substance, thiocyanate. Assuming an unchanged basal respiratory quotient, he calculated that during hyperventilation 21 to 47 per cent of expired nonmetabolic CO_2 came from outside the thiocyanate space, and, during CO_2 inhalation, 12 to 56 per cent of the CO_2 was retained outside this space. More recently Farhi and Rahn[25] have found in such experimental circumstances that two-thirds of the nonmetabolic CO_2 is gained or lost from a phase or phases outside the blood. Thus, it was clearly established that respiratory acid-base disturbances, consisting of deficits or excesses of carbonic acid, are buffered in body fluid phases other than the blood and the freely diffusible interstitial fluid, although the exact tissue sites of these "intracellular" buffer systems were not definitely identified.

RECENT STUDIES OF LINKED IONIC TRANSFERS IN WHOLE BODY BUFFERING

In the studies enumerated above, relatively little attention was paid to the obligatory exchanges of ions associated with the transfers of hydrogen. During the past 15 years the development of the techniques of flame photometry, body phase measurement by dilution of tracer substances, and tissue electrolyte analysis, has led to exploration of this complex subject. In addition, the observations of Darrow and his associates[19, 15] of the association of an extracellular metabolic alkalosis with experimentally produced deficiency of potassium, has focussed attention on the relationship of transfers of this predominantly intracellular cation to alterations in acid-base equilibrium. As a result much new light has been shed on ionic transfers associated with the functioning of the buffer systems of the body.

Acute respiratory disturbances of acid-base balance offer perhaps the best experimental preparation for the study of ionic transfers during buffering since no exogenous load of cations or anions is imposed. Such a study of acute respiratory alkalosis and acidosis in man has been carried out by the author and his colleagues[24] and may be used to illustrate some of the relationships involved. A brief account of these experiments will be pre-

TABLE 2. DISTRIBUTION OF WHOLE BODY BUFFERING OF ACUTE EXPERIMENTAL RESPIRATORY DISTURBANCES OF ACID-BASE EQUILIBRIUM

Investigators	*Experimental procedure*	*No. Expts.*	*Species*	*Av. distribution of buffering*		*Assoc. "intracellular" ionic tranfers*—av.*			
				Intravascular (red cell + plasma)	*Extravascular (interstit. + "intracell.")*	*Na*	*K*	*Anion*	*Extracellular reference space*
				per cent of total body buffering		equivalent per cent of total body buffering			
A. Respiratory Alkalosis:									
Brocklehurst and Y. Henderson[10]	Hyperventilation	5	Man	49†	51				
Irving, Ferguson, and Plewes[34]	"	4	Cat	3†	97				
Farhi and Rahn[25]	"	13	Dog	42†	58				
Giebisch, Berger, and Pitts[28]	"	5	Dog	38	62	+16	+4	—35 lactate)	Radiosulfate
Elkinton, Singer, Barker, and Clark[24]	"	6	Man	30	70	+37	—3	—36 (undeterm.)	Chloride
B. Respiratory Acidosis:									
Shaw[52]	CO_2 inhalation	6	Cat	12†	88				
Brocklehurst and Y. Henderson[10]	"	11	Man	54†	46				
Adolf, Nance, and Shiling[1]	"	6	Man	16†	84				
Irving, Ferguson, and Plewes[34]	"	4	Cat	2†	98				
Farhi and Rahn[25]	"	10	Dog	36†	64				
Giebisch, Berger, and Pitts[28]	"	4	Dog	34	66	—37	—14	+6 (lactate)	Radiosulfate
Elkinton, Singer, Barker, and Clark[24]	"	5	Man	35	65	—47	—9	+9 (undeterm.)	Chloride

* Exclusive of the red-cell phase.
† Underestimated since the "nonbicarbonate" buffering of hemoglobin and plasma protein is not included.

sented here, followed by a summary of similar experiments by other investigators.

One group of six normal human subjects hyperventilated, and a similar group inhaled 7.5 per cent carbon dioxide, for periods of approximately 30 minutes. The effects of this experimentally produced deficit or excess of carbonic acid on the ionic composition of several phases of the body fluids were assessed by assuming normal initial volumes for the phases of red cells, plasma, and interstitial fluid, equating the extracellular fluid with the chloride space, and deriving the internal exchanges of chloride, bicarbonate, hydrogen, sodium, and potassium, from changes in plasma concentration and from external balances (urinary excretion) of these constituents. The central principle of this quantitative analysis of buffer activity is that exchanges of hydrogen ion can be strictly accounted for from changes in extracellular and red cell bicarbonate which occur as the result of loss or retention of CO_2 through the respiratory tract.* This follows from the relationship:

$$HCO_3^- + H^+ \rightleftharpoons H_2CO_3 \rightleftharpoons CO_2 + H_2O$$

Red cell bicarbonate is included with the bicarbonate of plasma and interstitial fluid because of the red cell chloride shift. Thus, if one can measure or calculate the change in total amount of extracellular plus red cell bicarbonate (ΔHCO_{3er}^-) that takes place under a given set of circumstances, and correct this value for the amount of hydrogen retained (primarily bicarbonate lost) by the kidney (b_{H+})† and for the amount of hydrogen given up or taken up by the reciprocal change in protein nonbicarbonate buffers of the blood (ΔBuf_b^-),† then the balance must be equivalent, ion for ion, with the amount of hydrogen that must have been surrendered or retained by buffer systems in a phase or phases beyond the extracellular fluid (ΔH_i^+):

$$\Delta H_i^+ = \Delta HCO_{3er}^- + \Delta Buf_b^- + b_{H^+}$$

During 30 minutes of hyperventilation the average change in extracellular-red cell bicarbonate (ΔHCO_{3er}^-) in our subjects was —136 mEq. Since only 5 mEq. could be accounted for in the urine and 39 mEq. combined with hydrogen from the protein buffers of the blood, 92 mEq. of

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* See footnote * on page 192.

† In the absence of intake, the balance of hydrogen (b_{H+}) is calculated as minus the total of the urinary excretion rates of ammonium ion plus titratable acid minus bicarbonate, i.e., bicarbonate excreted with "fixed" cations represents hydrogen ion preserved to the body. Change in the non-bicarbonate buffer anions of blood, hemoglobin and plasma protein, can be calculated from the change in arterial pH. See text of original paper[24] for details of calculation.

bicarbonate must have combined with hydrogen from a source outside this phase to form H_2CO_3 for excretion as CO_2 through the lungs (Fig. 1A). Thus, ΔH_i^+ equalled —92 mEq. and represented $92/131^{sts}$ or 70 per cent of the extra-renal buffering of the respiratory acid-base disturbance imposed. Conversely, during CO_2 inhalation the average change in extracellular-red cell bicarbonate (ΔHCO_{3er}^-) was +32 mEq. Of the corresponding hydrogen ion released from the retained carbonic acid which was

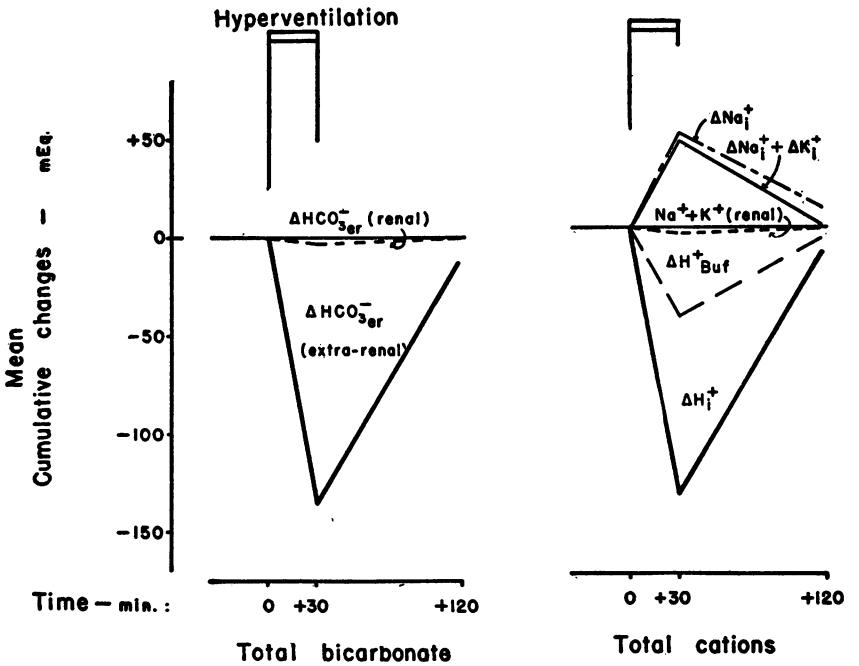

FIG. 1A. Acute respiratory alkalosis: the mean summated patterns of response of total extracellular bicarbonate and of total body cations, to hyperventilation.

Changes are plotted cumulatively from the onset to the end of the stimulus and to the end of the recovery period. On the left the dotted line indicates change in bicarbonate by renal excretion; the solid line indicates the total change in extracellular bicarbonate, the difference being extrarenal or respiratory. On the right are depicted the corresponding changes in cations. The dotted line represents renal excretion of sodium and potassium, the dashed line respiratory loss of hydrogen from blood buffer proteins, and the heavy line total hydrogen loss. The difference between the two latter represents transfer of hydrogen from total body cells. The associated changes in intracellular sodium plus potassium and in intracellular sodium alone are indicated above by the solid and dashed and dotted lines, respectively.

Loss of extracellular bicarbonate by the respiratory route greatly exceeds that by the renal. Associated with the loss of bicarbonate through the lungs is the hydrogen ion drawn in part from the protein buffers of the blood and in part from body cells. The latter decrement is partially replaced by transfer of sodium into cells. (Reproduced from the Journal of Clinical Investigation[24]).

the source of the bicarbonate, 12 mEq. were taken up by blood protein buffers but 2 mEq. were added by renal conservation; therefore, 22 mEq. of hydrogen appeared to have been transferred to buffers in phases other than the extracellular fluid (Fig. 1B). This value for ΔH_i^+ of +22 mEq. represents 22/34^{ths} or 65 per cent of the extra-renal buffering of the acid-base disturbance.

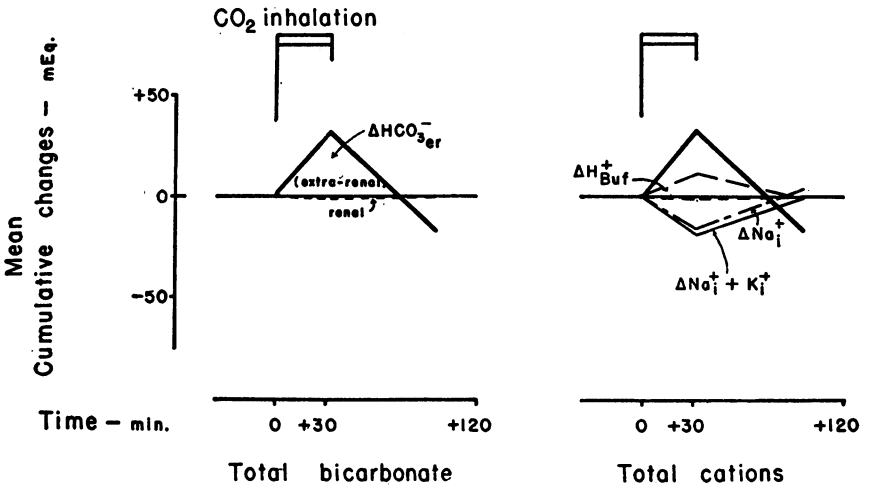

FIG. 1B. Acute respiratory acidosis: the mean summated patterns of response of total extracellular bicarbonate and of total body cations, to CO_2 inhalation. Data are presented as in Figure 1A.

Extracellular bicarbonate is increased in respiratory retention, and hydrogen is taken up by blood protein buffers and by body cells. Simultaneously the body cells lose sodium. (Reproduced from the Journal of Clinical Investigation[24]).

In this type of analysis, transfer of hydrogen ion in one direction cannot be differentiated from an equivalent movement of bicarbonate ion in the other direction; in either case the effect on acid-base equilibrium is the same. What does have to be answered, however, is the question: what are the associated transfers of other ions by which electroneutrality is maintained in the fluid phases involved? Obviously, as in the hyperventilating subject, hydrogen ion cannot enter, or bicarbonate ion leave, the extracellular phase without concomitant decrease in other cations or increase in other anions. Internal exchanges of sodium and potassium were calculated in these experiments by the usual method of multiplying the initial and final volumes of the extracellular phase (as equated with the chloride space) and correcting for the observed external balance of the respective ion. During hyperventilation, when 92 mEq. of hydrogen entered the "extracellular"

fluid from the nonextracellular or "intracellular" phase of the body, 48 mEq. of sodium moved in the opposite direction, potassium transfer was 4 mEq. in the same direction as hydrogen. Conversely, during CO_2 inhalation, when 22 mEq. of hydrogen appeared to have transferred into the "intracellular" phase, 16 mEq. of sodium and 3 mEq. of potassium moved in the opposite direction. The reciprocal relationship of change in "intra-

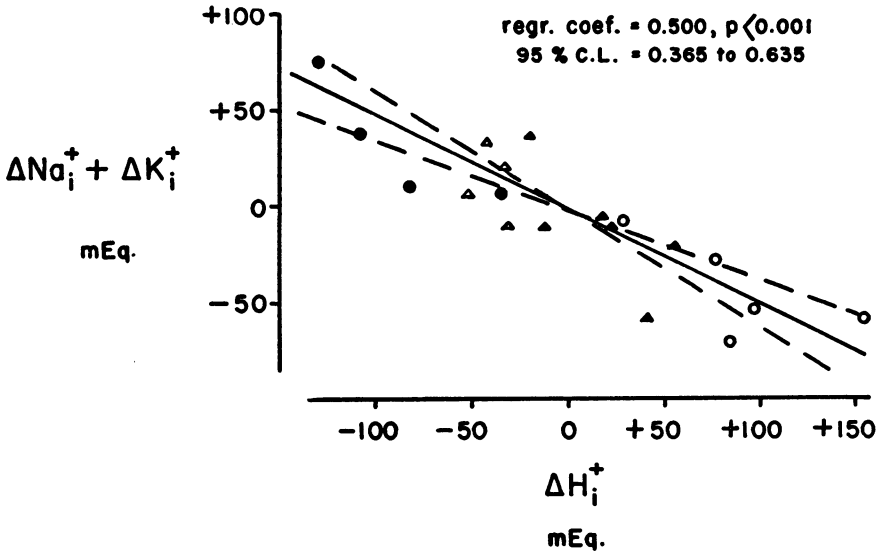

FIG. 2. Acute respiratory alkalosis and acidosis: relation of calculated transfers of cellular hydrogen to those of cellular sodium plus potassium. Solid circles (representing alkalosis) and triangles (representing acidosis) indicate changes during the period of stimulus; open circles and triangles indicate changes from the end of the stimulus to the latter part of the recovery period.

The inverse correlation between the two variables indicates a highly significant reciprocal relationship between transfers of these cellular cations ($r = -0.868$, $p < 0.001$). The relationship, as indicated by the regression coefficient (solid line), is approximately that of two hydrogen ions in exchange for one sodium or potassium ion. (Reproduced from the Journal of Clinical Investigation[24]).

cellular" hydrogen (ΔH_i^+) to the sum of the change in "intracellular" sodium and potassium ($\Delta Na_i + \Delta K_i$) in both experimental and recovery periods of all experiments is shown in Figure 2. The regression coefficient of —0.500 indicates a two for one exchange of hydrogen for sodium or potassium (the exactness of this reciprocal relationship is undoubtedly fortuitous). Since chloride shift was assumed to be restricted to the red cell: extracellular system, the transfer of some other anion with one of the hydrogen ions is the most likely possibility. As discussed below, the experi-

mental data of others indicate that this is probably an organic anion, mainly lactate.

In summary, our experiments are interpreted to show that in acute respiratory alkalosis or acidosis, induced in man for a period of approximately 30 minutes, 94 to 96 per cent of the response was that of extra-renal buffering and only 4 to 6 per cent was due to renal adjustments (the kidney simply had not had time to do more). Of the extra-renal buffering, 65 to 70 per cent was achieved by transfer of hydrogen across the "extracellular": "intracellular" phase boundary, in part in exchange for sodium (predominantly over potassium) and in part with an undetermined anion.

Our findings in humans are quite consistent with the results of the more elegant experiments in dogs of Giebisch, Berger, and Pitts[28] (Table 2). Their study was more definitive in that a greater respiratory acid-base stress was imposed, the renal component of response was eliminated by nephrectomy, and the internal ionic exchanges were calculated in reference to an independent measurement of the "extracellular" phase (as quantitated by the volume of distribution of radiosulfate). Their results may be summarized as follows: in respiratory alkalosis 38 per cent of the buffering could be accounted for by blood proteins (mainly hemoglobin with a chloride shift out of red cells), and 55 per cent by hydrogen transfer into the "readily available" extracellular fluid (35 per cent with lactate and 16 per cent in exchange for sodium plus 4 per cent for potassium transferred out of this extracellular phase). Conversely, in respiratory acidosis, 34 per cent of the buffering was accounted for by blood proteins (with a shift of chloride into red cells), and 57 per cent by transfer of hydrogen out of the "readily available" extracellular fluid (6 per cent with lactate and 37 per cent in exchange for sodium plus 14 per cent for potassium transferred into this extracellular phase). No definitive evidence was found by Giebisch *et al.* for a shift of chloride between the radiosulfate "extracellular phase" and intracellular fluids other than in the red cell. The results of the author and colleagues as enumerated above are surprisingly close to these of Giebisch *et al.*, which clearly define the major internal transfers involved in the whole body buffering of acute respiratory disturbances in acid-base equilibrium.

The exchanges of anions other than chloride between cells and extracellular fluid, under these circumstances, have long been recognized. Small shifts of phosphate were observed in the past[41, 45] and were found in the experiments of Giebisch, Berger, and Pitts cited above. An increase in plasma and extracellular lactate during the respiratory alkalosis of hyperventilation has long been known to be part of the buffering response to this acid-base stress.[2, 41] Recently, the quantitative data of Giebisch *et al.* on this

point have been reinforced by a report from Boucot, Lumb, Mahler, and Stanbury[9] to the effect that in acute respiratory alkalosis any fall in arterial plasma carbon dioxide in excess of 5 mM. per liter was accompanied by a nearly equivalent rise in plasma lactate; pyruvate rose as well and phosphate fell. These findings were interpreted to indicate that a glycolytic process is involved in the buffer activity of tissue cells.

Internal ionic exchanges which occur in the buffering of *acute metabolic disturbances* of acid-base equilibrium have also been the subject of recent experimental study (Table 1, A). When sodium bicarbonate was infused into nephrectomized dogs, Axelrod, Seip, and Pitts[3] observed that some 43 per cent of the alkali was buffered by ion exchange between the "extracellular" phase as measured by the volume of distribution of inulin and the remaining "intracellular" fluid; of this portion some 18 per cent was buffered by transfer of sodium with bicarbonate into the "intracellular" phase and 25 per cent by exchange of "intracellular" chloride for "extracellular" bicarbonate. Similar results were found by van Goidsenhoven, Gray, Price, and Sanderson[60] in a more chronic experiment in human subjects who ingested sodium bicarbonate for a period of days. Again, as calculated in reference to the inulin space, an average of 13 per cent of the retained sodium and bicarbonate transferred beyond this space and 37 per cent of the bicarbonate exchanged for chloride between the two phases. Singer, Clark, Barker, Crosley, and Elkinton[54] calculated the distribution of sodium bicarbonate acutely infused in hypertonic solution in man. At the end of two hours, of the retained bicarbonate (as buffer anion or its cationic equivalent), 26 per cent on the average was distributed in blood, 44 per cent in extravascular "extracellular" fluid, and 30 per cent in "intracellular" fluid. Since the ionic exchanges were quantitated in reference to an "extracellular" phase equated with the chloride space and calculated from the chloride balance, no exchanges of bicarbonate for "intracellular" chloride were derived; nevertheless, some two-thirds of the "intracellular" transfer of bicarbonate was accounted for by a simultanous net movement of cations in the same direction (35 per cent sodium in, minus 15 per cent potassium out). The problem of the identification of the "true" extracellular space in reference to which internal ionic transfers should be calculated is taken up later. However this phase is defined, it is clear that buffer systems in other phases of the body fluids are operative in acute metabolic alkalosis.

There is experimental evidence that such is likewise the case in *acute metabolic acidosis* (Table 1, B). Schwartz, Jensen, and Relman[48] administered ammonium chloride as an acidifying salt to human subjects previously depleted of sodium; 16 per cent of the retained hydrogen ion was

accounted for in the blood, 29 per cent in the extravascular "extracellular" fluid as defined by the chloride space, and 55 per cent in the remaining "intracellular" phase. The latter was associated with a loss from this phase of an equivalent 22 per cent of sodium and 34 per cent of potassium, the predominance of the potassium possibly being due to the sodium-depleted state of the subjects. Schwartz, Ørning, and Porter[49] investigated the proportionality of extracellular to intracellular buffering of hydrochloric acid infused into dogs in varying amounts. They found that, when time was allowed for equilibrium to be reached, the relative degree of buffering in each phase remained constant regardless of the load of acid imposed; no actual values for the buffer activity of the several phases were given. Swan and Pitts[58] studied the neutralization of hydrochloric acid infused into nephrectomized dogs; about 18 per cent of the hydrogen was buffered in the blood, 25 per cent in the remaining "extracellular" fluid as defined by the volume of distribution of radiosulfate, and 51 per cent in the "intracellular" phase by exchange for sodium (36 per cent) and potassium (15 per cent). In these experiments, in which an independent measurement of "extracellular" fluid was made other than with inulin, no definitive shift of chloride was observed other than with the red cell. Tobin[59] performed similar experiments in nephrectomized cats using both inulin and chloride as "extracellular" reference spaces. Using the inulin space, 35 and 5 per cent of the added hydrogen were exchanged for sodium and potassium, respectively, which entered the extracellular phase, and 20 per cent was transferred out of this phase with chloride; in respect to the chloride space, 58 per cent of hydrogen was exchanged for sodium and 6 per cent for potassium from outside this space. In both sets of calculations Tobin estimated that 22 to 24 per cent of the added hydrogen combined with bicarbonate (presumably expired) and some 14 to 16 per cent of the buffering was accounted for. Since this latter must include buffering by blood proteins and since part of the chloride shift undoubtedly took place into the red cell, the distribution between intravascular and extravascular phases of the total body buffering in these experiments is not presented in Table 1, B.

In summary, metabolic disturbances of acid-base equilibrium, like respiratory disturbances, are buffered by systems in multiple phases of the body fluids including one or more phases which lie beyond those ordinarily considered to be extracellular in location. Such buffering involves internal exchanges of sodium and potassium ions for hydrogen ions and, under some circumstances, chloride and/or other anions for bicarbonate. In this way electroneutrality is maintained, while hydrogen ion concentration is defended, in this heterogeneous phase system.

CURRENT PROBLEMS AND THE EVIDENCE FROM TISSUE ANALYSES

Despite the fact that investigations of this complex physicochemical and physiological phenomenon over the past half-century have amply confirmed and elaborated the concepts of Lawrence Henderson, many problems remain to be clarified.

The problem of quantitation of the total acid-base disturbance underlies the interpretation of all the data presented in the foregoing portion of this paper. Variation in method of this quantitation undoubtedly explains some of the discrepancies between the results as shown in Tables 1 and 2. In the study of respiratory disturbances of acid-base equilibrium, two main and different methods have been employed. One method has been to measure directly the total amount of carbon dioxide that has been retained or given up, i.e., the external balance of carbon dioxide, and to correct this value for its simultaneous metabolic production. This method is subject to error because of uncertainty as to this correction, uncertainty which is due both to the relative magnitudes of the two fractions of carbon dioxide and to possible variation in the basal respiratory quotient during the experimental stimulus. The other method has been to measure the change in amount of bicarbonate in the extracellular fluid (interstitial fluid plus plasma) and red cells and to calculate from this change the total buffer activity and the associated internal ionic transfers. This latter method must yield incorrectly low values to the extent of any change occurring in intracellular nonmetabolic bicarbonate; and Rosenbaum's[46] experiments cited above indicate that this may be a very significant moiety. Since the data of Giebisch *et al.*[28] and of the author and his colleagues[24] were derived by this second method, it is probable that the calculated proportion of intracellular buffering is underestimated in these studies.

The problem of identification of the extracellular phase that should be used as a reference point for calculation of transfer of "intracellular" ions is only too well known to every student of the field and does not need to be discussed in detail here. Recent evidence for a connective tissue sub-phase of extracellular fluid into which inulin penetrates much more slowly than does chloride or bicarbonate[40, 16] casts suspicion on ionic transfers, and especially on those of chloride, calculated in reference to the inulin space. On the other hand, the possibility that in at least some circumstances or in some tissues (such as skin) chloride may penetrate cells[20, 37] renders suspect ionic transfers calculated in reference to the chloride space. For these reasons, the interpretation of data concerning internal exchanges of ions must always take into account the particular "extracellular" reference phase employed in the derivation.

The characterization of *the buffering capacity and associated ionic transfers of specific organs or individual tissues* is a likely route to further clarification of this problem although the difficulty of the "extracellular" reference phase is still present. Despite the fact that the emphasis in this paper has been on the buffer response to acid-base stress of the total organism, experimental approach to the problem on the tissue level is not new. In 1928, for example, Fenn[26] compared the CO_2 dissociation curves of nerve and muscle with those of blood at different levels of CO_2 pressure and found the buffering capacity of those tissues to be one-third to one-half that of blood. The buffering of acidified blood by perfused hind limb of the dog by Banus and Katz[4] has already been referred to, as well as have the tissue analyses of muscle and bone under similar circumstances by Irving *et al.*[34] Bone particularly deserves to be investigated because of the recent evidence that at least part of the sodium in this tissue exchanges readily with that in extracellular fluid under a variety of circumstances that have been reviewed by Bergstrom[6] and by Nichols and Nichols.[39] In addition to exchanges of fixed cations of bone, carbon dioxide in the form of carbonate on the crystal surface may be transferable under conditions of acid-base stress. This is suggested by studies such as that of Irving and Chute[33] who found a loss of 6 to 14 per cent of bone carbonate in animals made acidotic with hydrochloric acid, and that of Bergstrom[5] who observed that the bones of acidotic rats lost carbonate and Na + K in equivalent amounts.

Data have been rapidly accumulating on changes which occur in the electrolyte content of skeletal muscle in acid-base disturbances. Of these the least well documented are the changes in intracellular bicarbonate and pH. Wallace and Hastings[42] made such measurements in the muscle of cats infused with strong acid or alkali; they found essentially no change in intracellular bicarbonate at the times when the extracellular concentration of the ion was elevated or depressed and estimated that the intracellular pH was conditioned mainly by changes in CO_2 pressure or H_2CO_3 concentration. On the other hand, Gardner, MacLachlan, and Berman[27] observed a decrease in intracellular bicarbonate, and hence pH, in rats experimentally depleted of potassium, a finding consistent with the hypothesis of intracellular acidosis in this condition (see below). More information is available concerning the distribution in muscle of sodium and potassium, under these conditions. Darrow and associates[18,19] found an increase in intracellular sodium in rats with metabolic alkalosis and a decrease in metabolic acidosis with reciprocal changes in cellular potassium. Cotlove *et al.*[17] calculated the fixed cation content of the intracellular phase of muscle in rats similarly treated. In the alkalotic rats, cellular potassium decreased and sodium increased but only in amounts equivalent to two-thirds of the

potassium lost, the total content of fixed cation (Na + K + Mg) being reduced; in the acidotic animals sodium decreased and potassium increased to a comparable extent in this phase. Levitt *et al.*[90] found that acidosis in rats mobilized sodium from bone cortex, tendon, and muscle with no significant changes in potassium content of the latter tissue. Tobin[97] in the experiments cited above and presented in Table 2 compared the data for the whole intact animal with those from muscle analyses in respect to shifts of sodium and potassium between the inulin space and "non-inulin" space; the loss of sodium from the "intracellular" phase of muscle accounted for two-thirds of the increase in "extracellular" sodium, the loss of "intracellular" potassium of muscle exceeded that found in the inulin space. Thus it is evident that the experimental data from tissue analyses are reasonably consistent with the data from studies of the intact organism in respect to heterogeneous phase buffering but are not nearly complete enough to define the exact rôle of buffer systems in specific tissues.

At this point it is necessary to comment on *the particular problem of the association of intracellular depletion of potassium with extracellular alkalosis;* does this phenomenon have some relationship to the internal ionic exchanges which are involved in whole body buffering of acid-base disturbances? This association, first described by Darrow and his co-workers,[19] has been amply confirmed in rats and in humans, but the factors which condition it have not been entirely clarified. Darrow's group showed[14] that it was not due simply to reciprocity of transfers of hydrogen and potassium in the renal tubule,[8] but that an internal shift of hydrogen with sodium into the "intracellular" phase of the body must be involved, a finding confirmed by Orloff, Kennedy, and Berliner.[42] Analysis of the electrolyte composition of rat muscle in respiratory acidosis by Cooke, Coughlin, and Segar[13] then indicated that in these circumstances of an elevated extracellular bicarbonate and total CO_2 content but depressed pH, intracellular potassium was if anything increased and intracellular sodium was diminished; cellular cation transfers therefore appeared to be related to changes in extracellular pH rather than to extracellular bicarbonate content. Nevertheless, potassium depletion of a severe degree may exist without a fixed relationship to intracellular sodium retention[47, 12] and without an accompanying extracellular alkalosis.[50, 56] In our laboratory,[56] normal human subjects maintained on a daily intake of potassium of less than 1 mEq. for as long as two to three weeks, and developing a total deficit up to 500 mEq. of the ion, have uniformly failed to show any signs of alkalosis even when high loads of sodium were given and but inconstantly when desoxycorticosterone was administered. Perhaps chloride subtraction as well as sodium load or adrenocortical hyperactivity, as suggested by Moore *et al.*,[38] is a necessary factor.

This problem is discussed in detail in a subsequent contribution in this volume.

In any case, the important point about this phenomenon in respect to whole body buffering, in the author's opinion, lies in the primacy of the stimulation. Given such a system of linked ionic transfers between multiple fluid phases, the precise transfers that take place depend upon the nature of the primary stimulus. If the primary event is the development of an extracellular alkalosis, respiratory or metabolic, hydrogen moves from the "intracellular" phase into the extracellular at least in part in exchange for sodium, and potassium transfers are minimal. Conversely, if the primary event is the development of an extracellular acidosis, the opposite occurs, namely, hydrogen moves into the "intracellular" phase mainly in exchange for sodium. Transfers of intracellular sodium appear to predominate over those of potassium in exchanging for hydrogen under these circumstances, despite experiments indicating that the extracellular *concentration* of potassium may be altered;[35, 51, 11] prior depletion of the organism of sodium[48] or potassium[17] may modify these cationic relationships. On the other hand, when intracellular potassium depletion is the primary event, extracellular hydrogen and sodium may transfer together into cells and so create or exacerbate a concomitant extracellular alkalosis rather than buffer or defend the hydrogen ion concentration of this phase. This concept of the linked ionic transfers which are involved in the body's defense of neutrality in its several fluid phases has been presented diagrammatically elsewhere.[23]

In this discussion little has been said of the rôles of the lungs and kidneys in the physiological regulation of acid-base equilibrium. Obviously, the physicochemical defense of neutrality by the buffer systems of the whole body is in itself a self-limiting process; continuous function of this process depends entirely on modification of these buffer systems by ionic exchanges with the external environment. Such modification by regulatory organs must therefore be the major determinant of acid-base equilibrium and of ionic composition in health and in states of disease, and the rôle of the kidney is discussed in the next paper. Nevertheless, the physicochemical process of whole body buffering is an integral part of the body's response to acid-base stress, and as such is well worth further investigation.

SUMMARY

The development of the concept of whole body buffering in multiple heterogeneous phases of the body fluids has been reviewed. Early studies of this phenomenon have been cited which indicated that a major portion of this physicochemical defense of body fluid neutrality took place in phases other than that of blood. More recent data have shown that such multiple

phase buffering involves a series of linked ionic exchanges. These exchanges include those of hydrogen, sodium, potassium, and certain anions, and result in the maintenance of electroneutrality in the face of redistribution of the hydrogen ion. Many problems remain in respect to quantitation of these processes, to definition of fluid phases, and to precise delineation of these exchanges in particular tissues such as muscle, nerve, and bone.

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